

Low temperature tolerance of soil arthropods — Some recent advances

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Summary :

Information on the effects of cold and freezing on soil arthropods and on their cold resistance mechanisms is reviewed. Recent studies on selected Arctic and Antarctic species is summarised comparatively. The occurrence of two survival strategies (freezing susceptible and freezing tolerant) is discussed, and the importance of an ecophysiological approach in such studies is emphasized.

Key words : freezing tolerance, freezing susceptibility, supercooling.

I. Introduction

The thermal tolerances of soil invertebrates have received increasing attention in the past decade, and adaptations to low temperature have been particularly investigated. Most attention has been given to terrestrial arthropods, and especially to the Insecta and Acari, which are major components of soil ecosystems throughout the world. Low temperature affects soil arthropods in a variety of ways, influencing their development and life cycles, their colonisation and survival of extreme environments, and their population and community dynamics. This paper summarises current information on low temperature tolerances of soil arthropods and focuses on the ecophysiological approach to problems in this field.

II. Effects of cold and freezing

Low environmental temperatures create two major stresses for soil arthropods by (i) reduction of activity, feeding and growth through cold, and (ii) increasing the probability of tissue freezing.

Cold shock results in a general metabolic deceleration as well as having reversible effects on cell membranes. Locomotory activity is reduced by cold (non-freezing temperatures) and results, eventually, in chill-coma, at which temperature mobility ceases. This is usually in the region of 5 to *ca* 15° C for temperate soil species, and may be as low as -8 to -11° C for polar forms. Although soil arthropods are immobilised during chill-coma, internal changes may occur which are normally completely reversible.

However, irreversible disturbances (cold injury) may occur above the freezing point (FPt) of the body fluids due to disruption of weak chemical bonds, damage to membrane lipids, etc. Such changes are likely to be brought about by desynchronisation of physiological processes arising from a lack of, or incomplete, acclimation (laboratory) or acclimatisation (field).

The body fluids (haemolymph, etc.) often remain in the liquid phase to temperatures well below their FPt, already depressed by dissolved solutes, a phenomenon termed supercooling (or undercooling). Many soil arthropods are able to supercool to

round -30°C to avoid freezing in nature. The temperature at which freezing occurs by rapid ice nucleation in the supercooled body is the supercooling point (SCP), and may be detected by a transitory increase in body temperature due to the latent heat of fusion during freezing.

The site of freezing is critical to soil invertebrates; extracellular ice being sustained whereas intracellular ice is usually lethal. Tissue ice brings about mechanical damage to cell membranes and reduces gaseous diffusion in and out of cells. The minimum critical cell volume is exceeded through dehydration and cell shrinkage, with membrane rupture and protein denaturation occurring. Concomitant with such effects, an increase in solute concentration may cause electrolyte imbalance leading to osmotic disruption and denaturation of membranes, enzymes, etc. Finally, there is the additional stress of ice recrystallisation during thawing from the frozen state.

III. Mechanisms of cold resistance

Ice nucleation may be homogeneous, involving only water molecules, or heterogeneous, when ice crystals form around a foreign body. Heterogeneous nucleation occurs in invertebrates down to *ca* -40°C , and many potential sources of nuclei occur in soil arthropods to promote freezing during supercooling. Two main strategies of cold resistance are utilized by poikilotherms: that of freezing susceptibility and freezing tolerance. Species using the former, resist tissue ice formation by supercooling and FPt depression, whilst those in the latter group freeze at relatively high sub-zero temperatures, often employing nucleators synthesized for the purpose.

The FPt and melting point (MPt) of haemolymph may occur at the same temperature (for arthropods in the zone 0 to *ca* -2°C) or the FPt may be depressed down to *ca* -10°C relative to the MPt. The latter situation is termed thermal hysteresis, and it has been shown that it is brought about by proteins (termed thermal hysteresis proteins or THP) (DUMAN, 1979a, b). THP may vary seasonally, thereby affording the arthropod protection during low temperature periods. THP may also act to stabilize supercooling over a wide temperature range in freezing susceptible animals (ZACHARIASSEN & HUSBY, 1982). The deepest extent of supercooling has been recorded as -63°C in a willow gall dipteran (MILLER & WERNER, 1980). It is generally recognised that supercooling is enhanced by polyhydric or sugar alcohols (polyols) in arthropods and other invertebrates, and glycerol is common in the species which have been investigated. In some freezing tolerant insects, ice nucleating proteins (INP) promote freezing by heterogeneous nucleation (ZACHARIASSEN & HAMMEL, 1976).

IV. Antarctic and Arctic studies

Freeze avoidance by extensive supercooling is a widespread survival strategy of polar terrestrial arthropods, especially in species which have a slower rate of develop-

ment due to the constraints of a low temperature environment (BLOCK, 1980). In the Antarctic, individual SCP distributions of six species of micro-arthropods showed a bimodality in that a high group (HG) at -10 to -15°C , and a low group (LG) at about -25 to -30°C occurred in most species during the year (BLOCK *et al.*, 1982). A small number of individuals in these populations die during winter, especially during rapid freeze-thaw cycles, which induce feeding and hence nucleation via gut contents. All the species showed a lowering of the mean LG SCP during winter associated with strong seasonal shifts in the SCP distributions. Five potential cryoprotectants were elaborated in these animals with glycerol being the most frequent in occurrence and in the highest concentration. In the cryptostigmatid mite, *Alaskozetes antarcticus*, increasing glycerol levels up to $ca\ 20\ \mu\text{g mg}^{-1}$ fresh weight were correlated with depression of the mean LG SCP from -25 to -35°C , and this in turn was reflected in changing glucose levels. All the species investigated at Signy Island, maritime Antarctic, except the collembolan *Parisotoma octodoculata*, possess a high capacity for supercooling and thereby ensuring that large proportions of their populations survive the extreme low winter temperatures (-20 to -28°C) in their soil habitats.

A survey of terrestrial arthropods shows supercooling to be a common form of cold tolerance, and SØMME (1982) concluded that insect eggs supercool slightly better than other life stages and other groups. Larval Diptera are amongst the high supercooling capacity arthropods, whilst some of the Araneida exhibit least supercooling.

By contrast, in nine species of Arctic insects, including Coleoptera, Diptera, Lepidoptera and Hymenoptera, six species proved to be freezing tolerant, the remainder were freezing susceptible (RING, 1981). Most insects showed the expected profiles of overwintering response during low temperature acclimation, although only one polyol appeared to be synthesized in any one species. In the freezing tolerant beetle larvae, *Pytho deplanatus*, from an alpine habitat, moderate glycerol levels and deep supercooling (to -54°C) were demonstrated. Whether this species is physiologically and biochemically incapable of synthesizing INP and natural nucleators are absent from the body in winter remains to be tested. Marked differences in the cold hardness of phylogenetically closely related forms suggest that the mechanisms of cold resistance have evolved independently on several different occasions. This contention is supported by the results of Antarctic micro-arthropod studies (BLOCK & SØMME, 1982; SØMME & BLOCK, 1982; BLOCK *et al.*, 1982).

Examination of the distribution of the two survival strategies throughout the terrestrial invertebrates (BLOCK, 1982) reveals that in 17 taxonomic groups, seven are freezing susceptible, four are freezing tolerant whilst six taxa contain representatives from both types. In the arthropods, the higher insect orders Lepidoptera, Diptera, Hymenoptera and Coleoptera contain species which have adopted either strategy.

V. Conclusions

Soil insects are the highest taxon and also the largest invertebrates to survive freezing in their natural habitats. Many of them survive freezing temperatures over long periods of time, and as their cellular organisation is as complex as any other metazoan, the study of such adaptations is of considerable theoretical and practical importance. In the union of ecological and physiological studies, there is a powerful tool for the solution of the more important problems in the field of poikilotherm cold tolerance. The adaptations of soil animals may be viewed as biological solutions to problems posed by their environment. In ecophysiology we are unravelling the answers which have been fashioned by natural selection during evolution.

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